QUANTITATIVE MEASUREMENT OF PERMEABILITY

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OSTERHOUT (8) has recently sought to explain the divergent results of some of his experiments and some of our own, on the basis of our confusion of permeability with absorption. It seems to us that any confusion that may have arisen is due largely to the different interpretations placed by different workers on such expressions as permeability in relation to complex systems like the cell. In this paper we discuss especially the meaning of the term permeability when it is used in a quantitative sense, and at the same time we take the opportunity of dealing with the points raised by OSTERHOUT in regard to the relation of his results and conclusions with our own. The term permeability may be classed with those expressions in current use in plant physiology which BARNES and LIVINGSTON (4) have described as cloaks for our ignorance. We may vaguely understand what is meant by the permeability of a membrane in regard to a particular substance, that is, its capacity for allowing the substance to pass through the membrane, although we may have no very clear idea as to how this takes place. In the case of the living cell, however, the matter is not so simple.

The nomenclature used in regard to the passage of substances into and out of the living cell has largely resulted from the work of DE VRIES on plasmolysis, and the theory derived from his results. It is a matter of common knowledge that as a result of the researches of DE VRIES (17, 18) and PFEFFER (9, 10), the plant cell came to be practically universally regarded as an osmotic cell, a solution surrounded by a semipermeable membrane, the plasma membrane, constituting the outer layer of the protoplast. On this view the permeability of the plasma membrane obviously means its capacity for allowing a substance to pass through the membrane. As plant physiology has developed, however, the realization of the complexity of the systems with which the plant physiologist has to deal has become more and more general, and it must be admitted that such a simple theory as that of DE VRIES will not afford a complete

explanation of the facts. Indeed, DE VRIES himself realized something of the complexity of the system, for he lays emphasis on the presence of two membranes which function in permeability phenomena, the outermost layer of the protoplasm, the plasma membrane, and the layer separating the rest of the protoplasm from the vacuole, the vacuole wall.[1]

In the simplest case of a plant cell immersed in a solution we have four phases: the external solution, the cell wall, the protoplast, and the vacuole; and in addition there are the limiting layers between these various phases which may have properties differing from those of either phase. We may represent such a system by the following scheme:

external solution	cell wall	protoplast	vacuole
phase boundary	phase boundary (plasma membrane)	phase boundary (vacuole wall)	

Again, in plant tissue intercellular spaces may also affect the results of investigations. Obviously in dealing with such a complex system the term permeability used in regard to the cell should only be used as a general expression to cover the various phenomena concerned in the passage of substances between living tissue and the external medium or between cell and cell in the living organism. It is in this sense that we have used the term permeability in our series of papers on these questions in *Annals of Botany;* we do not mean the capacity of substances to pass through any one particular phase of the system.

The permeability of living cells being then such a complex matter, it seems advisable not to use such expressions as "permeability coefficient," "measure of permeability," and "temperature coefficient of permeability," unless it is made clear what part of the system it is whose permeability is being considered. In our opinion the only legitimate use of such expressions is when they refer to the passage of substances into and out of the cell, or between one cell and another. Generally it is impossible by the methods of

[1] Cf. PFEFFER (11, p. 90): "In order to reach the cell sap a particle of water or dissolved substance must diosmose first through the cell wall and the plasmatic membrane which is closely applied to it, and finally pass through the internal limiting plasmatic membrane, which bounds the vacuole."

investigation at present available to analyze further the behavior of substances in passing through the various phases or across the boundaries between them. Hence, when we have used the term permeability in a quantitative sense we mean simply the capacity of a substance for entering the cell from the outside, or of passing out from the cell into the external medium, which are the phenomena with which we have so far mainly dealt. Generally we have not used the term permeability at all in a quantitative sense. Wherever possible it is much better to use the terms absorption or exosmosis, as the case may be, which have a definite unmistakable meaning and whose meaning does not depend upon an unproved and imperfect theory as does the term permeability as used by some writers.

In a paper (14) which appeared three years ago, we published the results of some experiments from which we concluded that the relation between time and absorption of hydrogen ions by potato cells was a logarithmic one, and that the temperature coefficient of this absorption was about 2.2. From this result it was pointed out that "the study of the effect of temperature on the absorption of the hydrogen ion would seem to indicate that this absorption is controlled by some chemical action in the cell, and is not the result of simple diffusion through the plasma membrane or of mere adsorption by the cell protoplasm." When therefore OSTERHOUT (8) says "it is evident, therefore, that the temperature coefficient observed by STILES and JØRGENSEN may be that of a chemical process involving the union of hydrogen ions with some constituent of the cell other than the plasma membrane," so far from contradicting our statement he is merely repeating our own conclusion in not very different words. When, however, he continues, "in which case it would have no bearing upon the problem of the nature of permeability," it would appear that he uses the term permeability, not in the general sense which we regard as the only legitimate one in which it can be used without qualification, but in a restricted sense, namely, the capacity of hydrogen ions for passing through "the plasma membrane (or other surface)." Against this restricted use of such a commonly used term as permeability we would enter a protest, as it rests upon a theory

which is unproved, which at best must be incomplete, and from which indeed many workers now dissent (FISCHER **1**, MOORE, ROAF, and WEBSTER **5, 6**). When, therefore, OSTERHOUT says of us that "they regard the temperature coefficient found by them as the temperature coefficient of permeability to hydrogen ions," he is completely misrepresenting our views on the matter. We never used the expression "temperature coefficient of permeability" for the reasons already mentioned, but if we had done so, we should certainly not have used the term permeability in the restricted sense in which OSTERHOUT appears to use it.

We may point out that OSTERHOUT's conclusion that we regard the temperature coefficient found by us as the "temperature coefficient of permeability" is based on the following assumptions: (1) that we "apparently reach the conclusion that 'the substance with which the acid reacts' is 'presumably the plasma membrane or some part of it'"; (2) that we support the view of PAULI and SZÜCS that the entrance of ions into the cell is due to the reversibility of a reaction between ions and the plasma membrane; (3) the title of our paper "The effect of temperature on the permeability of plant cells to the hydrogen ion." With regard to the first statement, we neither apparently nor in reality reached that conclusion. What we actually said was that our results indicated "that the quantity of substance with which the acid reacts, presumably the plasma membrane, or some part of it, remains constant as it does not influence the rate of the reaction." This is quite a different statement. We said "presumably the plasma membrane" because it could not be assumed that it was the plasma membrane;[2] it might be any part of the cell. It is quite an immaterial point; our argument holds equally whether the action takes place in the limiting layer or elsewhere in the cell.

Again, OSTERHOUT's second statement that we support the view of PAULI and SZÜCS is not founded on fact. We actually said, "this *suggests* that *either* the absorbing substance is present in such

[2] The term "plasma membrane" is another of those semimystical expressions whose use does not help in the elucidation of scientific problems. We prefer to use this expression in the way that LEPESCHKIN uses it, simply as meaning that part of the cell where the permeability phenomena are taking place. Compare our recent remarks on this term (**15**).

large quantity as compared with the acid that the amount changed is small in comparison with the total amount, *or* that the substance formed as a result of the absorption is broken down again almost as soon as formed. Such a view of the plasma membrane is held by PAULI and SZÜCS, who regard the entrance of ions into the cell as due to the reversibility of such a reaction between ions and the plasma membrane. *We feel, however, that more experimental evidence is required before such theories can be discussed adequately and with profit.*" It is extraordinary that anyone could see support for SZÜCS's view in that statement.

Finally, in the title of the paper the term permeability was used in its ordinary general sense, and in our opinion the title gave a reasonable representation of the contents of the paper, which should be its function.

For the reasons already stated we hold that that large body of workers who have included the absorption or exosmosis of dissolved substances among the phenomena of permeability are completely justified. OSTERHOUT's statements, "the results obtained by these methods have been so largely misinterpreted," and "the principal difficulty lies in confusing permeability with absorption" seem to be due to his giving to the term permeability an indefinite and yet restricted meaning. It is unfortunate that he should not have realized that he and the writers he criticizes use the word permeability in a different sense; it is still more unfortunate that he should attribute to them his own use of the term permeability, and it is particularly regrettable that he should assume they mean the same things by "temperature coefficient of absorption" and "temperature coefficient of permeability" (in his sense, not theirs) when they carefully avoid such an expression as "temperature coefficient of permeability" on account of its indefinite meaning.

OSTERHOUT says that he himself used a method for determining the temperature coefficient of permeability which is free from the "objections" just discussed. We may now consider how far this statement is justified. He states that "by this method the electrical conductivity of living tissue was determined in such a way that it may be regarded as a measure of the permeability of the protoplasm." We propose therefore to discuss OSTERHOUT's work under

three heads: (1) which part of the system it is, the permeability of which he intends to measure; (2) how far the values he obtains for the electrical conductivity of plant tissues are true measures of this conductivity; and (3) whether it is legitimate to assume that the electrical conductivity is a measure of the permeability.

In regard to the first question it is perhaps significant that when discussing the statements of the writers OSTERHOUT should speak of permeability in reference to the passage of substances through "the plasma membrane (or other surface)," while when discussing his own he should refer to the "permeability of the protoplasm." It is therefore not at all clear what it is that OSTERHOUT considers he is measuring, whether he is dealing with the whole cell content or part of it, or only the limiting layer of the protoplasm.

We come then to OSTERHOUT'S method of measuring the electrical conductivity of living tissues. The essential of this method (7) is that a pile of disks of *Laminaria* thallus is immersed in sea water or other medium between two electrodes. These are separated by a length of 20 mm. of sea water and the resistance between them measured. This resistance is called the resistance of the apparatus. The electrodes are then separated so that the roll of *Laminaria* disks is inserted between the electrodes in such a position that between each end of the roll of disks and the electrode is a length of 10 mm. of sea water. The resistance is again measured and the increase in resistance is taken to be the resistance of the tissue. Now whether the resistance of the tissue can be determined in this way depends entirely upon the form of the apparatus used, for the 20 mm. of sea water and the tissue must be strictly in series and there must be no surrounding conductor through which current might pass. As OSTERHOUT has never published any details regarding the arrangement of his apparatus, it is impossible to accept his results when their correctness is highly dependent upon the details of the experimental arrangement. Indeed, certain facts given in OSTERHOUT'S very inadequate description suggest an incorrect arrangement; for instance, why, if the sea water and *Laminaria* are arranged in series, should the resistance of 2 cm. of sea water be 305, while the resistance of 2 cm. of sea water plus a cylinder of sea water of the same transverse dimensions as the

tissue (5 cm. long) is only 392? No doubt an explanation of this is forthcoming, but it has not been given so far, and it will serve to indicate the necessity for a full description of OSTERHOUT'S apparatus and method before his conductivity measurements of tissues can be accepted by other workers.

Finally, there is the question as to whether the electrical conductivity of tissue can be used as a measure of permeability. Can it be assumed that the electrical conductivity as measured by KOHLRAUSCH'S method is really a measure of the permeability of the protoplasm to ions? We have already called attention (**12, 13**) to the fact that the conductivity of tissue is the resultant of the conductivity of a variety of different phases, and owing to the complex arrangement of these phases it cannot be assumed that the conductivity of the whole is the sum of the conductivity of each phase. HÖBER (**2, 3**), using a method which it is true is perhaps not above criticism, comes to the conclusion that the interior of the cell only contributes relatively slightly to the total conductivity. Moreover, OSTERHOUT neglects the fact that if the penetrability for ions increases, a necessary consequence of this may be increased diffusion between the external medium and the interior of the tissue, resulting in changes of concentration in the interior of the cell. Similarly, any change which altered the concentration or the distribution of free electrolytes in the interior of the cell would alter the conductivity. It may be, although we do not certainly know, that electrical conductivity gives a rough idea of the permeability of the cell; it is extremely unlikely that it gives numbers so exactly proportional to any kind of permeability that "temperature coefficients of permeability" can be calculated from them. Hence we consider it impossible to accept any of OSTERHOUT'S results obtained by his electrical conductivity method with *Laminaria* disks until (1) he makes clear what he means by permeability when this word is used in a quantitative sense; (2) he has given proof that his method does give values for the electrical conductivity of the tissue employed; and (3) he has produced evidence that the electrical conductivity of tissue can be taken as a measure of permeability in the sense in which he uses that word.

We should also like to raise two further points arising out of OSTERHOUT'S work. In the first place, we would point out that in

his discussion of our results, he would apparently apply conclusions derived from a brown alga immersed in a strong salt solution (about $\frac{N}{2}$), to potato tuber immersed in a dilute acid solution $\left(\frac{N}{1000}\right)$. Such a method of argument seems to us illegitimate. It is not to be accepted as a first principle that the permeability of every tissue, and permeability in regard to every substance or ion, will follow the same law. Secondly, we should like to caution in regard to temperature coefficients. When the temperature coefficient of the absorption of water by one tissue is found to be about 1.3 and by another tissue 3.0, as we have found with carrot and potato respectively, it should make one hesitate to draw conclusions as to the nature of a reaction from the magnitude of its temperature coefficient. That the temperature coefficient of the absorption of hydrogen ions by potato tissue is about 2.2 suggests, as we said previously, that the absorption is controlled by a chemical action, but without further evidence it is not more than a suggestion. This is forthcoming from the shape of the time-absorption curve and the fact that the absorption of hydrogen ions continues long after the concentration of hydrogen ion inside the tissue would be greater than that outside if no chemical action took place.

It must also not be forgotten that in cell problems we are dealing with a complex heterogeneous system, with probably a number of related and interdependent actions taking place, each one of which may have a different temperature coefficient. It would not be in any way surprising to obtain different coefficients for the same complex of processes with tissue that had had a different previous history, as we point out in a recent paper (**16**).

In conclusion, we should like to enter a plea for definiteness of statement and for the avoidance of semimystical expressions such as "permeability" or "plasma membrane" used in a quantitative and yet undefined sense. Above all, should be avoided the drawing of conclusions and the putting forward of theories on insufficient data.

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